Klinik für Kleintiermedizin
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Blood Compatibility Studies: New Insight into Canine Blood Types and Xenotransfusions in Cats

Inaugural-Dissertation

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1 Zusammenfassung

Vetsuisse-Fakultät Universität Zürich (2016)

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Studien zur Blutkompatibilität: Neue Erkenntnisse über canine Bluttypisierung und Xenotransfusion bei Katzen.

So wie der Mensch, haben auch Hunde und Katzen ihre eigenen Blutgruppensysteme, diese sind von klinischer Relevanz, wenn Individuen negativ sind für ein spezifisches Antigen auf den Erythrozyten und somit sensibilisiert werden können. Bei Hunden sind mehr als zehn Blutgruppensysteme beschrieben worden, aber routinemäßige Blutgruppenbestimmung, wurde aufgrund der limitierten Verfügbarkeit von Reagenzien anderer Blutgruppen nur für DEA 1 angeboten. Bei Katzen wird das AB Blutgruppensystem als das Bedeutendste angesehen. Während Katzen klinisch relevante anti-A und anti-B Alloantikörper besitzen, müssen Hunde durch eine vorherige Bluttransfusion sensibilisiert werden.
Die zwei hier präsentierten Forschungsarbeiten, adressieren neue Aspekte der caninen Bluttypisierung und der Kreuzprobe bei Feliden: (1) Durch die Verwendung von monoklonalen Antikörpern wurden Hunde für DEA 1 und die zwei neuen Bluttypen Kai 1 und Kai 2 überprüft. Weiter wurden die unterschiedlichen Expressionen von DEA 1 sowie die gängige Kai 1 und seltene Kai 2 Expression bei 500 Hunden aus Nord-Amerika dokumentiert. (2) Die Dokumentation von Kompatibilitätsproblemen bei Xenotransfusionen bei Katzen, die nach Erhalt von caninen Erythrozyten, hämolytische Transfusionsreaktionen erfuhren und durch Heterotypisierung und-Kreuzproben, die Inkompatibilität von caninem und felinem Blut gezeigt haben. Diese beiden Studien sollen dazu beitragen, die Transfusionsverfahren in der Kleintiermedizin weiter zu verbessern.

Stichworte: Alloantikörper, Dog Erythrocyte Antigen, Hämolytische Transfusionsreaktion, Blutgruppenbestimmung, Kreuzprobe.

1.1 Summary

Vetsuisse-Fakultät Universität Zürich (2016)

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Blood Compatibility Studies: New Insight into Canine Blood Types and Xenotransfusions in Cats.

Just like humans, dogs and cats have their own blood group systems which can be clinically important as individuals negative for a specific red cell antigen may become sensitized. In dogs >10 blood group systems have been described, but routine typing has only been offered for DEA 1, due to the limited availability of reagents for other blood groups. In cats the AB blood group system is considered most important. While cats have clinically important anti-A and anti-B alloantibodies, dogs need to be sensitized by prior transfusion to develop alloantibodies.
The two original research studies address novel aspects of canine blood typing and feline crossmatching: (1) Utilizing monoclonal antibodies we surveyed dogs for DEA 1 and the two new red cell antigens Kai 1 and Kai 2 and documented the varied expression of DEA 1 as well as common Kai 1 and rare Kai 2 expression among >500 dogs in North America, and (2) We report on xenotransfusions in cats receiving canine red blood cells experiencing acute hemolytic transfusion reactions and showing in heterotyping and -crossmatch studies the incompatibility of canine and feline blood. These studies will further improve the transfusion practice in small animals.

Keywords: Alloantibody, Dog Erythrocyte Antigen, hemolytic transfusion reactions, blood typing, crossmatching.

2 Survey of two new (Kai 1 and Kai 2) and other blood groups in dogs of North America

Euler CC, Lee JH, Kim HY, Raj K, Mizukami K, Giger U. J Vet Intern Med., in press, 2016.

2.1 Summary

Background: Based upon serology, >10 canine blood group systems have been reported.

Objectives: We surveyed dogs for dog erythrocyte antigen (DEA) 1 and 2 new blood types (Kai 1 and Kai 2), and some samples also were screened for Dal and DEA 3, 4 and 7.

Methods: Blood samples provided by owners, breeders, animal blood banks, and clinical laboratories were typed for DEA 1 by an immunochromatographic strip technique with a monoclonal antibody and analysis of band intensity. Both new antigens, the Dal and other DEAs (except DEA 7 by tube method), were assessed by a gel column method with either monoclonal or polyclonal antibodies. The same gel column method was applied for alloantibody detection.

Results: Of 503 dogs typed, 59.6% were DEA 1+ with 4% weakly, 10% moderately and 45.6% strongly DEA 1+. Regarding Kai 1 and Kai 2, 94% were Kai 1+/Kai 2-, 5% were Kai 1-/Kai 2- and 1% were Kai 1-/Kai 2+, but none were Kai 1+/Kai 2+. There was no relationship between Kai 1/Kai 2 and other blood types tested. Plasma from DEA 1-, Kai 1-, Kai 2- dogs, or some combination of these contained no detectable alloantibodies against DEA 1 and Kai 1 or Kai, respectively.

Conclusions: The new blood types, called Kai 1 and Kai 2, are unrelated to DEA 1, 3, 4 and 7 and Dal. Kai 1+/Kai 2- dogs were most commonly found in North America. The clinical relevance of Kai 1 and Kai 2 in canine transfusion medicine still needs to be elucidated.

2.2 Introduction

Each blood group system represents allelic surface red blood cell (RBC) antigens (types) that differ among individuals with >1% positive or negative dogs observed within a population. Alloantibody production can occur in individuals missing ≥1 of these antigens. Since the 1960s, ≥ 10 canine blood group systems have been reported.[1-4] Because dogs do not appear to have any naturally-occurring alloantibodies, these blood types were originally defined after experimental or accidental clinical sensitization of dogs by transfusions.[3,5] In the mid-1970s, a workshop committee of the International Society for Animal Blood Group Research (now known as International Society of Animal Genetics) assigned 7 blood groups with the dog erythrocyte antigen prefix dog erythrocyte antigen (DEA).[6,7] Subsequently, additional blood groups have been discovered, with Dal being the most recent and important,[8, a,b] but they have not received an official designation. Moreover, it is difficult to determine wheter all of them are indeed new, because the original cells and typing antisera are no longer available. None of the canine blood group systems have been defined at the protein or molecular level thus far.[1,3,4]

Clinically acute hemolytic transfusion reactions only have been reported in previously transfused dogs and only against DEA 1.1, 4 and Dal or unknown blood types.[2,3,9,10] The DEA 1 blood group system, initially described with 3 types, DEA 1.1, 1.2 (and likely 1.3 [A3]), recently has been found, utilizing an anti-DEA 1 monoclonal antibody, to be a complex autosomal dominant allelic system, with a DEA 1- type and varied degrees of DEA 1 positivity from 1+ to 4+.[11,12] In South Korea, additional blood group systems utilizing 2 new monoclonal antibodies, anti-Kai 1 and anti-Kai 2, currently are being investigated. The monoclonal anti-Kai 1 and anti-Kai 2 antibodies are of the IgM and IgG class, respectively, and recognize different antigens of RBC membrane proteins in immunoblot studies. These monoclonal antibodies were utilized in the investigations reported here.

We surveyed the prevalence of 3 blood group antigens in a large group of dogs from North America with monoclonal antibodies against DEA 1, Kai 1 and Kai 2 and in a subset also compared those results with available antisera for other blood groups. Our results identified (1) the degree of DEA 1 positivity in a large canine population, (2) the presence of Kai 1 and Kai 2 in North American dogs, (3) the lack of a relationship of 2 new canine blood types, Kai 1 and Kai 2, to other blood group systems, and (4) the absence of alloantibodies in any type negative dogs before receiving transfusions.

2.3 Materials and Methods

2.3.1 Animals and Samples

Canine blood samples were obtained from owners, breeders, and blood banks or were made available as residual samples from the Clinical Pathology Laboratory at the Ryan Veterinary Hospital and were studied at the PennGen laboratory, University of Pennsylvania, from March to December 2015. Most samples originated from Philadelphia and the surrounding Tristate area (Pennsylvania, New Jersey and Delaware) except for the Greyhounds and Dalmatians. The >1 mL ethylene-diaminetetraacetic acid (EDTA)-anticoagulated blood samples were kept chilled and typed within 10 days of collection. To standardize results, 20% and 1% RBC suspensions were prepared for each sample as previously described.[11] The few DEA 7+ and DEA 7- blood samples were typed by ABRI.[c] There were no specific selection criteria, but rather the samples that could be made available as previously typed by ABRI. The anti-DEA 7 antibody reagents were very weak and did not work satisfactorily in our hands. These studies were performed and approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

2.3.2 DEA 1 Blood Typing by Immunochromatographic Strip

For DEA 1 typing, a commercially available immunochromatographic strip technique[d] was used according to the manufacturer's instructions and as described previously by the PennGen laboratory, adjusting blood samples to a 20% RBC suspension.[11,13] The results were captured by imaging,[e] and the DEA 1 band strength was assessed on a scale of 0 (no band) to 4+ (as strong as control band) by visual and densitometric analyses by GeneTools software[f].

2.3.3 Kai 1, Kai 2, Dal, and DEA 3 and 4 Blood Typing by Gel Column (Gel)

Blood samples were centrifuged to pellet RBCs. Plasma was removed and stored frozen. The packed cells were washed 3 times with phosphate-buffered saline (PBS), each time discarding the supernatant. Then, 10 µL of packed cells were added to a culture tube[g] with 1 mL low-ionic strength solution[h] to prepare a 1% RBC suspension. We adapted the original tube assay for Kai 1 and Kai 2 to a gel column technique. Thereby, plain saline gel columns[i] were used to detect agglutination reactions as previously described.[14] Briefly, 25 µL of antibody reagent

(Kai 1, Kai 2, Dal provided by MC Blais, Montreal University) and DEA 3 and 4[c] for extended typing (as well as saline as autocontrol), and 50 µL of 1% RBC suspension were added on top of the gel and incubated at 37°C for 15 minutes in the manufacturer's incubator.[j] Thereafter, the cards were centrifuged for 15 minutes at 85 x *g*, with the manufacturer's centrifuge.[k] The degree of agglutination strength was graded from negative (0: all RBC at the bottom of the gel) to positive (4+: all RBC at the top of the gel). Results were interpreted as negative if ≤1+ with practically all cells pelleted.

2.3.4 Alloantibody Detection in DEA 1-, Kai 1-, Kai 2- or Both Dogs' Plasma

For detection of alloantibodies in plasma samples, we used the gel as previously described.[8,14] Briefly, we prepared 2-4 plain saline gel columns for each sample by placing 25 µL of plasma from Kai 1- or Kai 2- dogs and adding 50 µL of Kai 1+ or Kai 1-, and Kai 2+ or Kai 2- RBCs in LISS, respectively. The results were graded as positive (majority of RBCs at the top of gel) or negative (majority of RBC at the bottom of the gel) as done with gel typing results above.

2.4 Results

This large typing survey included 503 dogs from North America representing 80 breeds and mixed breed dogs. A larger number of Dalmatians (N=108 related to a separate Dal typing study), Greyhounds (70, mostly blood donors), and mixed breed (60) dogs were blood-typed, whereas all other breeds only had 1-25 dogs (median, 6) represented. Overall, there was no significant difference in blood type frequencies among breeds with at least 20 dogs typed, except for Dal- dogs (S. Goulet, U. Giger, C.C. Euler, M.C. Blais, unpublished data, 2016). The typing survey results are summarized in Tables 2.1 and 2.2.

Table 2.1. Patterns of DEA 1, Kai 1 & Kai 2 typing results among 503 dogs from North America.

Dogs #	Dogs %	DEA 1	Kai 1	Kai 2
282	56.0	+	+	-
191	38.0	-	+	-
15	3.0	+	-	-
10	2.0	-	-	-
3	0.6	+	-	+
2	0.4	-	-	+
Positive Results				
#		300	473	5
%		59.6	94.0	1.0

Table 2.2. Canine typing results of DEA 1, 3, 4, and 7 and Dal related to Kai 1 and Kai 2. Results < 1+ are graded as negative.* External DEA 7 typing results.

	DEA 1		DEA 3		DEA 4		DEA 7*		Dal	
	+	–	+	–	+	–	+	–	+	–
Kai 1 + and Kai 2 –	282	191	1	79	133	0	15	8	5	5
Kai 1 – and Kai 2 –	15	10	5	7	24	1	0	0	5	5
Kai 1 – and Kai 2 +	3	2	0	2	4	0	0	0	0	5
Dogs #	503		94		162		23		25	

2.4.1 DEA 1 Typing and DEA 1 Alloantibodies

Utilizing the Strip with a monoclonal anti-DEA 1 antibody and grading the DEA 1 band strength identified 40.4% DEA 1- dogs, with the remainder having varied degrees of DEA 1 positivity. Adjusting the PCV to 20% and quantitative analyses of the DEA 1 band strengths permitted differentiation of the degree of DEA 1 positivity (Fig 2.1).

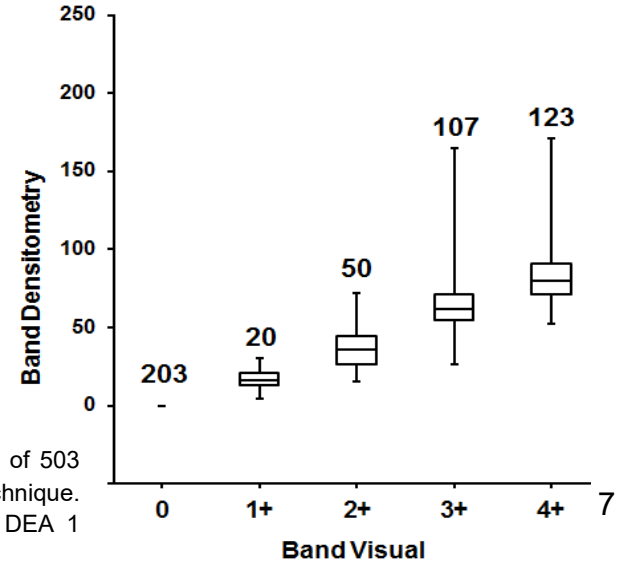

Figure 2.1. Visual and densitometric DEA 1 analyses of 503 dogs with immunochromatographic strip typing technique. Median, range and extremes for each densitometric DEA 1 band strength compared to visual band assignment.

A large proportion of the DEA 1+ samples showed a very strong band (3+/4+), and only a small proportion exhibited 1+ and 2+ reactions. There was a close correlation between semiquantitative visual and densitometric assessment of the degree of DEA 1 positivity differentiating DEA 1- and weakly, moderately, and strongly DEA 1+ dogs. None of DEA 1- (and not previously transfused) dogs tested had any observable DEA 1 alloantibodies by gel.

2.4.2 Kai 1 and Kai 2 Typing and Alloantibodies

Monoclonal antibodies against red cell antigens were developed in South Korea, and 1 anti-Kai 1 and 1 anti-Kai 2 antibody (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016) were used in the survey reported here. We established a simple blood typing technique utilizing gel saline columns and adjusting the canine RBC quantity (1%) to compare the degree of the agglutination reactions (Fig 2.2). The majority of canine blood samples typed Kai 1+ with most showing strong (3+/4+) and few moderate (2+) and weak (1+) agglutination reactions with the anti-Kai 1 reagent. In contrast, nearly all dogs typed as Kai 2- with only 5 dogs typed strongly Kai 2+, respectively (Table 2.3). In addition, 5% of the dogs were Kai 1- and Kai 2-, but none were positive for both Kai antigens. Assessment of the plasma from all Kai 1- and Kai 2- dogs indicated no alloantibodies against Kai 1 and Kai 2 cells, respectively.

Table 2.3. Agglutination reactions for Kai 1 and Kai 2 typing with gel column. The degree of agglutination shows grading from negative (0: all of RBC at the bottom of the gel) to positive (4+: all of RBC at the top of the gel).

	# Dogs	
Agglutination	Kai 1	Kai 2
0	30	498
1+	0	0
2+	2	0
3+	76	0
4+	395	5

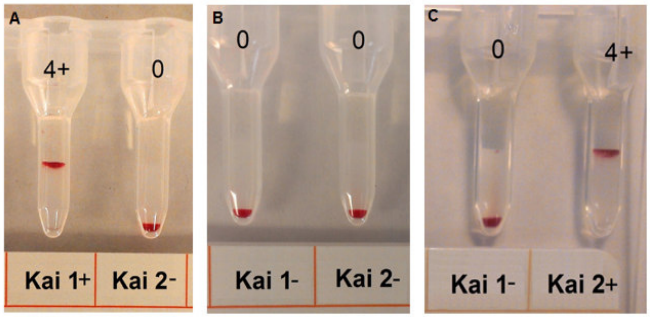

Figure 2.2. Gel column typing results showing the different Kai typing patterns for Kai 1 and Kai 2. (**A**) Kai 1+/Kai 2-, the most common pattern; (**B**) Kai 1-/Kai 2-; and (**C**) Kai 1-/Kai 2+. All autocontrols were negative for agglutination.

2.4.3 Extended Typing for DEA 3, 4 and 7 and Dal

To determine wheter there was a relationship between the new Kai blood types and other blood groups, a subset of dogs were further typed by another laboratory for DEA 7 (in our laboratory the available DEA 7 did not identify any agglutination reactions) or further typed for DEA 3, 4 and Dal with the Gel and available polyclonal antisera from negative dogs sensitized with positive red cells (Table 2.2). All dogs typed were DEA 4+ with 1 exception; this sole DEA 4- dog was a Dalmatian and typed as Kai 1-, Kai 2-, DEA 1+, DEA 3+, and Dal+. No relationship between DEA 1, 3, 4, and Dal and Kai 1 and Kai 2 could be detected. Furthermore, no relation between DEA 7 and Kai 1 could be found. Specifically, there were Kai 1- dogs that were DEA 1+, DEA 3+, DEA 4+ or Dal+. Similarly, the few dogs that were Kai 2+ could be DEA 1-, DEA 3- or Dal-.

2.5 Discussion

Two monoclonal antibodies against red cell antigens recently were developed in South Korea. Based on their original preliminary evaluation, these 2 murine antibodies, named anti-Kai 1 and anti-Kai 2 (Kai refers to dog in Korea), recognize 2 different canine red cell antigens (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016). To determine wheter Kai 1 and Kai 2 exist in North America and are novel red cell antigens, we compared their expression to those of known blood types. We documented the presence of both Kai 1 and Kai 2 in North America. As far as we could determine, neither Kai 1 nor Kai 2 is associated with each other (albeit we did not find any Kai 1+/Kai 2+ dogs) nor with any known canine blood group system. Both Kai 1- and Kai 2- dogs as well as DEA 1- dogs lacked any naturally-occurring alloantibodies against Kai 1 and Kai 2 and DEA 1, respectively. The clinical importance of these new blood groups in transfusion treatment still remains to be determined.

In our survey of 503 dogs mostly from Philadelphia and the Tristate area, including a relatively large number of Greyhounds (70 samples) and Dalmatians (108 samples) from the USA and Canada, 94% of dogs were Kai 1+ but only a few (1%) were Kai 2+. Therefore, the combination of Kai 1+ and Kai 2- was most frequently (94%) observed. The Kai 1+/Kai 2- blood type constellation was found in every blood donor Greyhound tested, whereas in other breeds tested, Kai 1+ and Kai 1- dogs were found. Among the few Kai 2+ samples, 3 were from Lhasa Apsos. Since completion of these studies, a limited preliminary survey from the United Kingdom also found several Lhasa Apsos with Kai 1-/Kai 2+ blood (Watson, C.C. Euler and U. Giger, unpublished data, 2016). Similarly, a recent preliminary survey of mastiffs in South Korea indicated a 17% Kai 2+ frequency (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016). Another possible constellation found was Kai 1-/Kai 2-, whereas no dog was Kai 1+/Kai2+. Results of limited family studies are consistent with an autosomal dominant trait for Kai 1 (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016). Because the frequency of the 2 phenotypes Kai 1+/- and Kai 2+/- are <99% and ≥1%, respectively, Kai 1 and Kai 2 should be considered blood groups rather than high- or low- frequency (public or private) red cell antigens.[15,16] DEA 4 was thought to be a blood group, although ≥99.5% of dogs seem to be DEA 4+ and thus, it should be considered a high frequency antigen. In contrast, Dal was thought to be a high-frequency red cell antigen with Dal- originally only found in a few Dalmatians but larger surveys found Dal- dogs in Dobermans and other breeds and thus, it should be recognized as a blood group.[a,b,8-10]

Blood typing for Kai 1 and Kai 2 was determined by traditional tube agglutination assays in South Korea. We adapted the gel column technique for Kai 1 and 2 typing and alloantibody studies with neutral NaCl gel column cards, which can be better standardized and are simple to perform and interpret, as we have previously shown for DEA 1[11, 17] and other DEA types[14] as well as Dal types.[a,b] The degree of agglutination was very strong (3+ or 4+) for both Kai 1 and Kai 2 antigens in nearly all cases with a few samples giving weaker reactions (2+). Moreover, the results were stable during repeat typing, as previously shown for DEA 1,[11] and results were easy to interpret. However, we did have a few cases in which we observed an unexplained split reaction, with the majority of cells located on top of the gel despite having a few RBCs pelleted; we designated such cases as 4+. These dogs were neither previously transfused nor had any illness. Overall, these monoclonal antibodies and current gel column typing (and tube) techniques seem well suited to detect positive agglutination reactions for screening dogs for the Kai 1 and Kai 2 blood types in clinical settings. Commercial Kai typing kits may be developed in the future, if there is more clinical evidence for blood incompatibilities and transfusion reactions.

The DEA 1 blood group system has not been defined at the protein and molecular level, but based upon recent studies seems to originate from a single locus with $\geq$4 alleles, and thus DEA 1- and weakly to strongly DEA 1+ dogs rather than DEA 1.1, DEA 1.2 and DEA 1.3 types.[1,2,11] We have shown previously that DEA 1 typing with a monoclonal antibody by gel column, flow cytometry, and chromatographic strip produced identical results.[11] In this survey of 503 dogs, we applied the chromatographic strip method with visual and densitometric grading at PCV 20% as previously described.[11] The proportions of DEA 1- and strongly DEA 1+ (3+/4+) dogs were far larger than those that were weakly (1+) or moderately (2+) DEA 1+. Among breeds with at least 25 dogs represented, DEA 1- and DEA 1+ dogs were found, which is consistent with our prior smaller surveys[11,12] and also reports with DEA 1.1 and 1.X polyclonal antisera.[18] However, our survey included a biased group of dogs, because it included many Greyhound blood donors from 2 animal blood banks, where DEA 1- dogs are preferred, and many Dalmatians related to a separate Dal typing survey (S. Goulet, U. Giger, J. Arsenault, A. Abrams-Ogg, C.C. Euler, M.C. Blais, unpublished data).[a,b] Finally, consistent with prior observations,[3] plasma from the previously untransfused DEA 1- dogs screened indicated a lack of naturally-occurring anti-DEA 1 alloantibodies.

Results of the Dal typing survey of the dogs in our study will be presented elsewhere, but as expected based upon small prior surveys, Dal- dogs were found among Dalmatians and Doberman Pinschers, Shi Tzus as well as in few other breeds (S. Goulet, U. Giger, J.

Arsenault, A. Abrams-Ogg, C.C. Euler, M.C. Blais, unpublished data, 2016).[a,b] However, these frequencies of Dal- dogs may not be representative because the number of dogs tested per breed was small and owners and breeders of Dal- dogs likely submitted samples from related dogs in order to find potential blood donors for their Dal- dogs. Again, the Dal blood group system has no relationship to the new Kai 1 and 2 blood group systems.

Comparison of the DEA 1 typing with Kai 1 and Kai 2 typing results in our survey indicates that these are likely different blood group systems. Reagents are no longer available for all previously reported canine blood group systems, such as DEA 5, 6 and 8. These DEA canine blood groups and alloantibodies had not well been characterized (mostly based upon agglutination reactions),[13, 16] and consequentially, it will be impossible to determine whether the antigens Kai 1, Kai 2 or both are related to any previously described blood groups. However, based upon our limited extended typing comparisons, there is no relationship to the known DEA blood groups for which reagents were available (DEA 1, 3, 4, 7, and Dal). Thus, Kai 1 and Kai 2 appear to be newly discovered red cell antigens. Moreover, a direct relationship between Kai 1 and Kai 2 could not be determined, although there were no dogs positive for Kai 1 and Kai 2. However, a few dogs were Kai 2+ and based upon immunoblotting studies utilizing the 2 monoclonal antibodies, the sizes of the 2 red cell antigens are different (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016).

These DEA and Kai 1 and Kai 2 typing surveys and alloantibody studies have clinical implications. From a practical perspective, it is highly advantageous that most dogs are either clearly DEA 1- or strongly DEA 1+ and similarly clearly negative or positive for either Kai 1 or 2 type. The occasional weakly to moderately DEA 1+ dog is still important to identify. Currently, it is not known wheter weakly to moderately DEA 1+ cells will induce alloantibodies in DEA 1- recipient dogs and wheter these weakly to moderately DEA 1+ dogs would be sensitized by strongly DEA 1+ blood. Thus, specific evidence-based recommendations regarding those cannot be made yet. We continue to follow the cautious practice of typing donor and recipient, giving DEA 1- and also weakly DEA 1+ recipient dogs only DEA 1- blood, and labeling weakly or moderately DEA 1+ donors as DEA 1+ donors.[3, 11] Recommendations for Kai must await demonstration of any alloantibody development after transfusions. Preliminary unpublished experimental studies show the development of anti-Kai 1 and anti-Kai 2 alloantibodies in Kai 1- and Kai 2- dogs, respectively (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016).

The lack of naturally-occurring alloantibodies against DEA 1 and both Kai antigens further supports the practice of not crossmatching before a first transfusion.[3] Although acute

hemolytic transfusion reactions have been documented in previously transfused dogs due to DEA 1, DEA 4 and Dal incompatibilities,[2, 8-10] there is no published evidence of alloantibody production and acute hemolytic transfusion reactions due to Kai mismatched transfusions. Indeed, we used monoclonal murine antibodies against Kai 1 and Kai 2 and not anti-Kai alloantibodies from previously transfused dogs for typing. However, unpublished clinical results after Kai mismatched transfusions indicate the development of anti-Kai 1 and anti-Kai 2 alloantibodies and hemolytic reactions (J.H. Lee, U. Giger, H.Y. Hee, unpublished data, 2016). Therefore, it appears appropriate to follow up dogs with incompatible crossmatches for Kai-type mismatches and potentially select donors based also on the Kai types in previously transfused dogs.

In conclusion, we established a simple and accurate gel column typing technique for Kai 1 and Kai 2 and found both red cell antigens with most dogs to be Kai 1+/Kai 2- in North America. These blood types represent novel and distinct blood groups with potentially different frequencies among breeds. Dogs negative for Kai 1, Kai 2, or both do not have any naturally-occurring alloantibodies in their plasma. The clinical importance of Kai 1 and Kai 2 in canine transfusion medicine needs to be determined.

2.6 Acknowledgements

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2.7 Footnotes

[a] S. Goulet, MC Blais, ACG Abrams-Ogg. Prevalence of the Dal Blood Type in Doberman Pinschers and in Canine Blood Donors. Proceedings of the ACVIM Congress, Nashville, TN; June 2014

[b] S. Goulet, MC Blais. Production and characterization of anti-Dal antibodies following sensitization of a Dal -negative dog. Proceedings of the ACVIM Congress, Indianapolis, IN; June 2015

[c] ABRI, Stockbridge, MI

[d] "Strip"; Alvedia, Lab Test DEA 1, Limonest, France

[e] Syngene G:Box, Syngene USA, Frederick, MD

[f] Syngene USA, Frederick, MD

[g] Fisher Scientific, Pittsburgh, USA

[h] LISS, ID-Diluent 2, DiaMed GmbH, Cressier, Switzerland

[I] "Gel"; BioRad ID-Cards, NaCl, Enzyme Test and Cold Agglutinins DiaMed

[j] ID-Incubator 37 S I, DiaMed-ID, Microtyping System, DiaMed GmbH, Cressier, Switzerland

[k] ID-Centrifuge 12 S II, DiaMed-ID, Microtyping System, DiaMed GmbH, Cressier, Switzerland

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3 Xenotransfusion of anemic cats with blood compatibility issues

Euler CC, Raj K, Mizukami K, Murray L, Chen CY, Mackin A, Giger U. Vet Clin Pathol. 45:244-53,2016.

3.1 Summary

Background: Finding compatible feline blood donors can be challenging.
Canine blood has been occasionally used when compatible feline blood was not available in emergency situations.
Objectives: The study goals were to describe the effects of xenotransfusion in 2 anemic cats receiving canine blood because of discordant blood types and acute transfusion reaction, respectively, and to report in vitro heterotyping and crossmatching results between canine and feline blood samples.
Material and Methods: Blood samples from patients and other cats and dogs were typed, crossmatched, and assessed for alloantibodies using gel, card, and immunochromatographic strip techniques.
Results: Cat 1 was found to have type AB blood. Cat 2, which experienced an acute transfusion reaction, had type A blood. Neither had detectable alloantibodies against feline RBC. Both cats transiently improved after transfusion with canine blood; however, acute intravascular hemolysis occurred and the PCV rapidly declined. Blood typing post xenotransfusion with DEA 1 strips revealed a positive control band that was absent in feline blood, thus allowing for the identification of transfused canine RBC. Longitudinal assessment revealed that canine RBC could no longer be detected 4 days after xenotransfusion.
Major crossmatching (feline plasma with canine RBC) resulted in both positive and negative reactions, depending on the cat. Minor crossmatching results showed mostly incompatibility.
Conclusion: While both cats survived xenotransfusion, the positive control band on the DEA 1 strip revealed that transfused canine RBC were short-lived and intravascular hemolysis occurred. Crossmatch results between cats and dogs showed varied incompatibilities and may not predict transfusion reactions.

3.2 Introduction

Because of the natural occurrence of strong anti-A and varied anti-B alloantibodies, feline patients and blood donors are blood typed for the AB blood group system and/or crossmatched in order to assure blood type compatibility. Several AB blood typing kits are available for in-clinic and laboratory use, although occasionally typing difficulties occur. Additional blood type (eg, Mik) incompatibilities and acute hemolytic and other transfusion reactions have been observed, particularly in previously transfused cats.[1] Crossmatching is particularly indicated after prior transfusions, but is not generally performed for a first transfusion.[2-7]

Transfusions are far less frequently performed in cats than in dogs for various reasons, including fewer clinical signs associated with anemia, fewer bleeding problems, the need to sedate donor cats, and difficulties in collecting and processing blood. When AB-matched feline blood is unavailable and conditions are dire, xenotransfusion (ie, transfusion of blood from one species to another species) with canine blood has been attempted, particularly in Australia and France.[8-11] A recent review of canine blood transfused to cats[12], mostly based upon 1960s experimental studies[8,9,13], and a few anecdotal clinical reports[14,15], claimed compatible crossmatch results between dogs and cats, and clinical improvement of anemic cats receiving canine blood for the first time. Clinical improvement was seen despite the very short life span of transfused cells. However, fatal reactions occurred when a second transfusion was given after 7−8 days. Those authors concluded that canine blood could be given for a first transfusion when feline blood was not available.[12] Alternatively, a highly purified polymerized bovine hemoglobin solution may be considered under such circumstances, although this product is currently only available in Europe.[16]

Here, we report on 2 anemic cats receiving canine RBCs because of discordant AB blood typing results and an acute transfusion reaction to feline blood, respectively. The mixing effects of canine and feline blood were assessed in typing and crossmatch tests.

3.3 Animals and Methods

3.3.1 Blood samples

Small (0.5−1 mL) EDTA anticoagulated blood samples were available from 2 anemic cats that received one therapeutic canine packed red blood cell (pRBC) transfusion. One fresh whole blood transfusion from a canine blood donor at the ASPCA Animal Hospital, New York, another at the Animal Health Center, College of Veterinary Medicine, Mississippi State University Starkville, MS, respectively, and several other blood donors were studied at PennGen Laboratories, School of Veterinary Medicine of the University of Pennsylvania, Philadelphia, PA (PennGen). Medical records and laboratory test results from both feline patients were reviewed.

Additionally, left-over EDTA anticoagulated blood samples from ill and healthy cats and dogs, submitted to the Clinical Pathology Laboratory at the Ryan Veterinary Hospital, University of Pennsylvania, were evaluated at PennGen. All samples were obtained and analyzed with approval of the University of Pennsylvania institutional animal care and use committee.

3.3.2 Blood typing techniques

Samples were typed at PennGen using 3 methods according to the manufacturers' instructions and published methods with minor modifications briefly described below.

3.3.3 Immunochromatographic Typing Strips

("Strip"; Alvedia, Lab Test A+B and DEA 1, Limonest, France)[4,17]

Briefly, 3 drops of diluent (Buffer, canine or feline) were placed into wells of a 96-well plate. Ten microliters of washed RBC suspension (PCV 20%) were mixed with the diluent. An immunochromatographic strip (impregnated with monoclonal antibodies) was then dipped into the RBC suspension for approximately 2−3 min until the RBC had migrated to the other end of the strip. The results were interpreted as follows: a red band at the position marked C (control) must appear for result interpretation (note exception when performing heterotyping, because control reagent reacts species- specifically [see below]), and the presence of a visible red band at the position marked DEA 1 (for canine typing) or A and/or B (for feline typing)

indicated the expression of the antigen on RBC membranes, respectively. The band intensity was then further measured as follows: 0, no visible band; 1+ very faint band; 2+ light band; 3+ bright red band; 4+ intense red band. Any visible bands of 1+ or stronger were considered as positive.[17]

3.3.4 Agglutination Typing Card

("Card"; DMS Laboratories Inc., RapidVet-H, Flemington, NJ, USA)[3,4]

Briefly, one drop of diluent was placed into the 3 wells marked autocontrol, A, or B on a feline blood typing card, and 3 wells marked autocontrol, positive control, and DEA 1 on a canine blood typing card, respectively. The reagents were rubbed off the well surface into solution with a wooden stick. Then, one drop of EDTA anticoagulated blood was added to each well, and the card was carefully agitated. The results were read after 1 min as follows: 0, no agglutination; 1+, many small agglutinates; 2+, some larger agglutinates and many small ones; 3+, a few large agglutinates; and 4+, 1 large agglutinate. Visible agglutination reactions of 2+ or stronger were considered as positive.

3.3.5 Gel Column

("Gel"; BioRad ID-Cards, NaCl, Enzyme Test and Cold Agglutinins DiaMed GmbH, Cressier, Switzerland) [3,4,18]

Ten microliters of RBC pellets (washed 3 times with phosphate buffered saline [PBS], each time discarding the supernatant) were added to 1000 µL of low-ionic strength solution (LISS) to prepare a 1% RBC solution. A volume of 25 µl of one of the following reagents: anti-A, anti-Mik, or anti-DAL serum (PennGen), Triticum vulgaris lectin (anti-B; Sigma Co., LLC, St Louis, MO, USA), anti-DEA 4 serum (Animal Blood Resources International, Dixon, CA, USA), or recipient serum (for backtyping and crossmatching) were pipetted on top of the gel columns. Then, 50 µL of the RBC solution were loaded on top of the gel. Gel columns were incubated for 15 min at 37°C and then centrifuged for 15 min at 85*g* in the manufacturer's incubator and centrifuge, respectively. The distribution of RBC in the gel column was graded as follows: 0, all RBC at the bottom of the gel; 1+ a few RBC agglutinates in the lower half of the gel, but the large majority of RBC at the bottom of the gel; 2+, RBC agglutinates

throughout the gel, 3+, RBC throughout the gel as well as on top of the gel; and 4+, all RBC on top of the gel column. A grading of ≥ 2+ was considered a positive test result.

3.3.6 Crossmatch Tests

For major and minor crossmatch and backtyping assays, the gel column method was applied as described above for typing, but using plasma instead of typing reagent.[18]

3.4 Results

3.4.1 Case reports

Cat 1

Following skin debridement and neutering, a young adult, male domestic shorthair cat, weighing 3.3 kg and testing negative for feline leukemia virus (FeLV) antigen and *feline immunodeficiency virus (*FIV) antibody, started oozing immediately from the surgical sites and developed marked anemia by the next day at the ASPCA Animal Hospital, New York, NY. A CBC revealed a severe nonregenerative normocytic and normochromic anemia and mild thrombocytopenia, without evidence of intravascular hemolysis. The PT and APTT were infinitely prolonged. An acute blood loss anemia due to severe coagulopathy, presumably due to ingestion of an anticoagulant rodenticide, was diagnosed.

The cat was treated with vitamin K_1 (10 mg SC, SID) and wound compression. In-clinic blood typing tests indicated type B blood with the Card, while the Strip revealed type AB blood. The apparent discordant blood typing results and lack of readily accessible type-compatible feline pRBC led to xenotransfusion with 64 mL of canine pRBC (DEA 1-) given in divided doses as 3-4 hour infusions, with the initial portion of the first transfusion given as a rapid bolus due to the cat's critical condition.

Within 1 h of initiating transfusion, the cat started eating and grooming. The mucous membranes turned slightly pink after 4 h, the oozing stopped, and no overt adverse reactions were observed any time after the transfusion. The next day (Day 1), the PCV was 21%, but the plasma appeared hemolyzed, with a plasma hemoglobin level of 2.7 g/dL (Figure 3.1A, B). Repeat PT and APTT test results on Day 1 were within the normal range. While the cat's PCV declined close to pretransfusion levels (14%) by Day 4, the cat did clinically well and did not show any further bleeding. The wounds healed well over the next 3 weeks. Furthermore, the PCV returned to the normal range and the coagulation tests remained normal (Figure 3.1A).

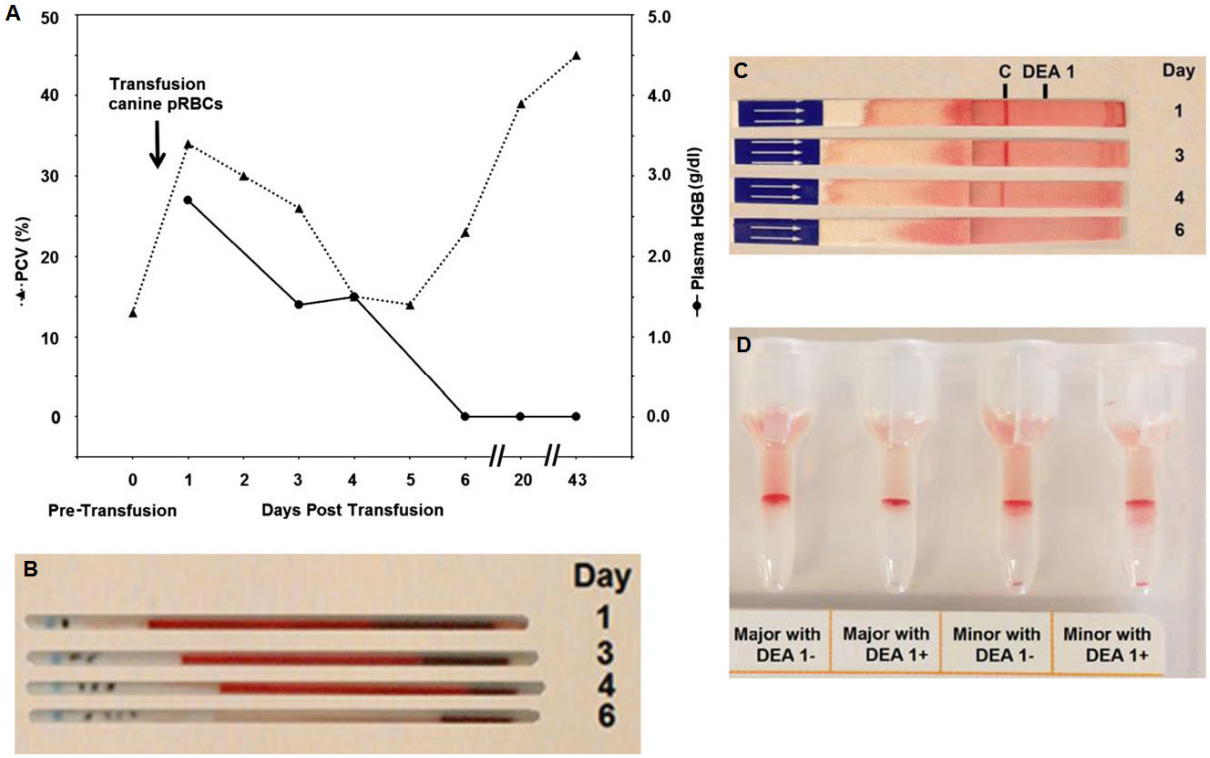

Figure 3.1. Illustrative results from Cat 1, which received a xenotransfusion with canine pRBC. (**A**) PCV and plasma HGB values (On day 0 the plasma
appeared nonhemolyzed). (**B**) Microcapillary tubes showing PCV decline and hemolyzed plasma. (**C**) DEA 1 Strip showing a fading positive control band.
(**D**) Hetero-crossmatch Cat 1, 20 days post transfusion, showing incompatibility for the major and minor crossmatch with DEA 1+ and 1- blood.

Cat 2

A 10 –month-old, FeLV- and FIV-, male domestic shorthair cat weighing 4.1 kg, with no history of prior transfusion, was presented with a severe nonregenerative normocytic-normochromic anemia (PCV 6% [Figure 3.2A] and 0% reticulocytes) to the Animal Health Center, College of Veterinary Medicine, Mississippi State University, Starkville, MS. The in-clinic Card indicated type A blood. After transfusing ~1 mL of feline blood from a known type A donor, the recipient cat exhibited acute bradycardia, open mouth breathing, hypothermia, and collapse, and the transfusion was immediately stopped. Subsequent major crossmatch test results between patient plasma and the original donor blood used and 2 other feline blood donors, both also typed as type A, were strongly incompatible using a commercial gel tube crossmatch kit (Companion Animal Crossmatch Major, DMS Laboratories Inc., RapidVet-H). Repeated blood typing, using the Card, confirmed that the recipient and all donors tested had type A blood.

The cat recovered uneventfully from the acute transfusion reaction with supportive care (intravenous fluids, flow-by oxygen and warming). The inexplicable acute transfusion reaction paired with initially incompatible crossmatch test results led to a xenotransfusion the following morning (12 h after the transfusion reaction) with 60 mL of fresh whole blood over 4 h from a canine donor (DEA 1-, DEA 4+, DEA 7+). The cat showed no overt clinical reaction. The PCV was 22% shortly after the transfusion, but the plasma appeared hemolyzed over the following days. Four days after the initial transfusion, the PCV fell to 7%, and the cat developed open mouth breathing, bradycardia and collapse, again necessitating emergency supportive care as described previously. A littermate was located, found to have type A blood and be compatible to the patient on major and minor crossmatch using the commercial gel tube crossmatch kit. Sixty milliliters of fresh whole blood from the FeLV- and FIV- littermate were transfused over 4 h without any transfusion reaction. The recipient cat stabilized shortly after starting the transfusion with the littermate's blood and the PCV rose to 15%. While a cause for the severe nonregenerative anemia could not be identified, the cat was treated twice daily with oral doxycycline (25 mg), prednisolone (7.5 mg) and cyclosporine (25 mg). The cat recovered uneventfully, with a PCV of 22% and 31% at discharge on Day 7 and recheck on Day 13, respectively, on a tapering course of prednisolone and cyclosporine (Figure 3.2A).

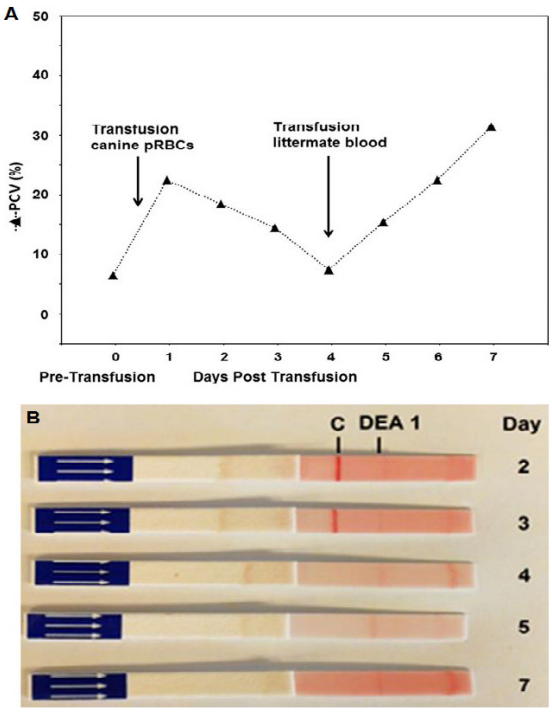

Figure 3.2. Illustrative results from Cat 2, which received a xenotransfusion with canine whole blood. (**A**) PCV values. (**B**) DEA 1 Strip showing a fading positive control band.

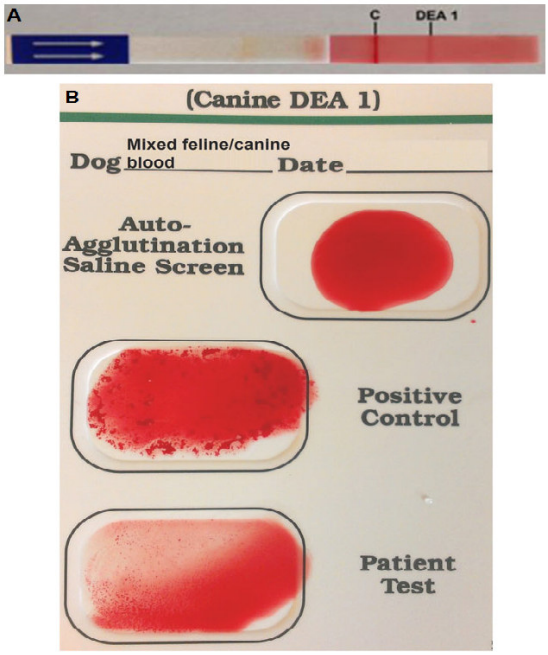

Figure 3.3. Mixture of feline and canine blood tested with canine DEA 1
blood typing Strip (**A**) and Card (**B**). Strip and Card showed regular reactions with the positive control band and well, respectively.

3.5 Follow-up blood typing studies of transfused cats at PennGen

3.5.1 Feline typing

Pre- and posttransfusion blood samples from Cat 1 consistently typed as AB by Strip and Gel, but typed as B by Card. Furthermore, DNA testing revealed a heterozygosity for the B allele which is consistent with having the other allele for the AB type (Laboklin, Bad Kissing, Germany).[19] Retyping Cat 2 and the initial incompatible feline donor, the 2 other feline donors and the littermate donor by applying different techniques consistently showed type A blood. All of these cats also typed as Mik+.

3.5.2 Canine Typing

When using the DEA 1 Strip with posttransfusion blood from both cats, no or only faint bands were detected at the DEA 1 site, which was expected as DEA 1- blood was transfused to both cats. However, a strongly positive control band was noted on Day 1 post transfusion with samples from both cats, which completely disappeared by Day 3−4 (Figure 3.1C and 3.2B), respectively.

3.5.3 Feline major crossmatch and alloantibody studies

In major crossmatch tests, plasma samples from both anemic cats were compatible with RBC from 4 type A cats. Neither cat had any detectable anti-A or anti-B alloantibodies in their plasma by Gel.

3.5.4 Major crossmatch with other canine red blood cells

A pre-transfusion plasma sample from Cat 1 showed no agglutination reaction with canine RBC by Gel; no pretransfusion sample was available from Cat 2. In contrast, strong agglutination reactions (3+/4+) were noted between plasma from both Cat 1 (Figure 3.1D) and Cat 2 and RBC from dogs at PennGen at every time point tested post transfusion.

3.5.5 Feline and canine typing and crossmatching studies with other cats and dogs

In order to better assess the reactions of feline blood typed by the DEA 1 Strip and Card, and crossmatch test results of feline blood with canine blood by Gel, we performed additional typing and crossmatch/mixing studies (Figure 3.3A, B). None of the 20 feline blood samples tested showed a positive control band on the DEA 1 Strip, but all of them showed a weak (1+) to moderate (3+) DEA 1 band (Table 3.1; Figure 3.4A). Moreover, testing feline blood samples using the DEA 1 Card, produced negative to moderate (3+) agglutination reactions in the positive control as well as in the DEA 1 well (Table 1; Figure 4B).

Table 3.1. Heterotyping of feline and canine blood with Strip and Card.Canine typing with AB Strip was negative, both for the AB Band and Control. All cats tested were blood type A.

	Band Strength (0 to 4+) with DEA 1 Strip	
# Cats	DEA 1 Band	Control Band
3	1+	0
11	2+	0
6	3+	0
	Agglutination Strength (0 to 4+) with Card	
# Cats	DEA 1 Well	Control Well
7	1+ to 3+	0 to 1+
4	1+ to 4+	2+
12	1+ to 3+	3+ to 4+

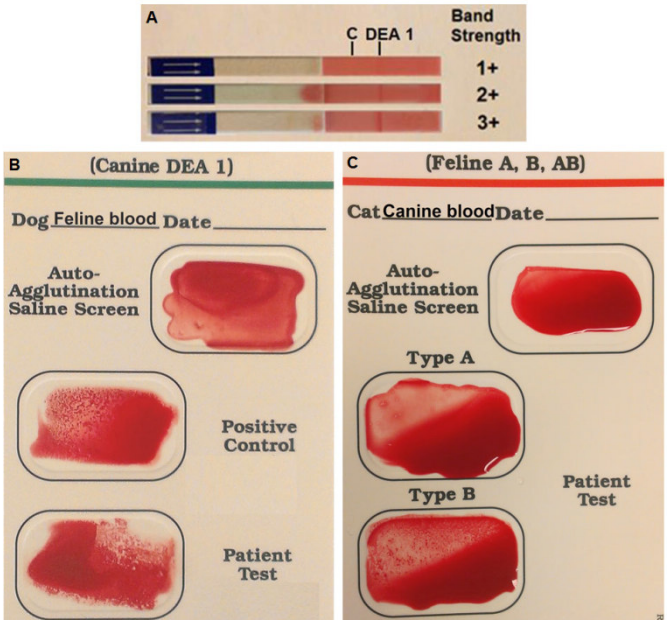

Figure 3.4. Heterotyping feline and canine blood with Strip (**A**), and Card (**B**). Canine typing with Card showed weakly positive (1+/2+) agglutination in the B well but no reaction in the A or Control well (**C**).

Crossmatching 20 feline control samples (16 type A and 4 type B cats) individually with 5 DEA 1+ and 5 DEA 1- canine blood samples showed both positive and negative results (Table 3.2; Figure 3.5). However the major crossmatch of each individual cat was either consistently compatible or consistently incompatible with all canine donor RBC. Further crossmatching of Cat 1 with 2 DEA 1+ and DEA 1- dogs on Day 20 and 43 post transfusion revealed strongly positive major and minor crossmatch results. The minor crossmatch was incompatible with most feline plasma and canine RBC combinations. Finally, typing of 10 canine blood samples with the feline AB Strip showed no reaction at the A, B, or control band sites. Typing these canine samples with the feline AB Card showed no reaction in the A well, but consistently mild agglutination reactions in the B well (Figure 3.4C).

3.6 Discussion

This report describes two anemic cats that received canine RBC as a xenotransfusion on an emergency basis because of issues with feline blood typing and compatibility.

The cats eventually recovered clinically despite acute hemolytic transfusion reactions following transfusion with canine blood. Monitoring the control band on DEA 1 typing strips allowed tracking of transfused canine RBC and revealed that these cells were very short-lived. Testing heterocrossmatches between feline patients and canine donors revealed both compatible and incompatible major crossmatch results and frequent agglutination reactions in minor crossmatch studies independent of AB and DEA 1 blood type, respectively. As our results indicate that canine DEA 1- RBC are rapidly cleared following transfusion into cats and our heterocrossmatch studies indicate that compatibility of canine blood cannot be assured in feline patients, xenotransfusions should be avoided except when compatible feline blood and ultrapurified polymerized bovine hemoglobin cannot be located in time. Based upon our hetero-crossmatch studies, results seem to be mostly incompatible and do not assure compatibility of canine blood in feline patients.

Table 3.2. Heterocrossmatch by Gel of 20 cats with 5 different DEA 1- and 5 different DEA 1+ dogs (see also Figure 5). Minor heterocrossmatch tests by Gel showed strongly incompatible results independent of the canine DEA 1 blood type and few crossmatch compatible reactions (data not shown).

Cats		Major Crossmatch with	
Type	*n*	DEA-1- (*n* = 5)	DEA 1+ (*n* = 5)
A	5	0 to 1+	0 to 1+
	1	1+ to 3+	1+ to 3+
	2	0 to 2+	0 to 2+
	1	2+ to 4+	0 to 4+
	7	2+ to 4+	2+ to 4+
B	2	2+ to 3+	1+ to 4+
	1	0 to 2+	+2 to 3+
	1	0	0

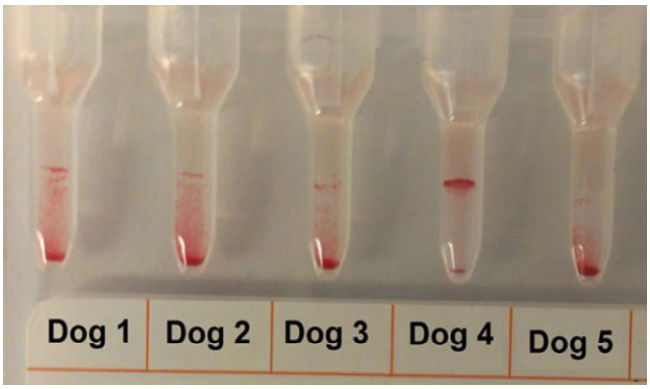

Figure 3.5. Heterocrossmatch of feline plasma and canine RBC by Gel. Plasma from 1 feline type B cat mixed with DEA 1+ RBC from 5 dogs showing negative and positive agglutination reactions.

While several feline AB typing kits are available for clinical practice, typing of AB and B cats can still pose challenges. Erroneous and discordant blood typing results have been previously reported in cats, and can occur as a result of human error in performing the test and interpreting results, as well as damage to and limitations of test kits used.[3,4,20] Detection of the

rare type AB is a particular concern as the Card does not recognize all type AB cats and may wrongly type them as type B as noted in Cat 1 of this report (the type A blood of Cat 2 was correctly typed). Furthermore, some FeLV+ anemic cats have reportedly been typed as AB when actually having type A blood.[4] Both cats of this report were FeLV-, but other yet to be identified diseases may affect RBC antigen expression. We have previously recommended having type AB results confirmed in a reference laboratory, as was done with Cat 1 in this report. We have also recommended that type B results be confirmed by backtyping, because every type B cat older than 12 weeks has strong anti-A alloantibodies (kittens may also have anti-alloantibodies after colostrum ingestion).[21] Neither adult Cat 1 nor 2 in this report had any detectable anti-A or anti-B antibodies.

While AB blood typing of both recipient and donor cats is generally recommended and performed prior to transfusion in clinical settings, crossmatching has been much less likely performed before a first transfusion in cats, although a recent study indicated that ensuring that both typing and crossmatching results are compatible prior to a first transfusion in cats may serve to maximize posttransfusion hematocrits.[22] Clearly, if AB blood typing is not available, a crossmatch test as simple as mixing a drop of plasma with whole blood should be used, as the strong anti-A alloantibodies in type B cats readily detects any AB incompatibility.[2] However, the detection of other blood type incompatibilities requires RBC washing (a more cumbersome procedure), technical skills at performing the test, and expertise in interpreting results. An in-practice commercial gel tube crossmatch kit was used for Cat 2, but its reliability has thus far only been evaluated for dogs (Villarnovo D, et al., V.D., unpublished data).[5]

Beyond the AB blood type, there is little information about other feline blood groups and RBC antigens. The Mik antigen is quite common, as very few Mik- cats have been found, but Mik- cats may have naturally occurring alloantibodies. However, typing for Mik is restricted, because of a lack of typing reagents.[1] Incompatible major crossmatches are readily observed following prior and repeat transfusions, supporting the presence of additional unknown RBC antigens.[23] Cat 2 had not been transfused previously, but was initially found to have an apparent incompatible major crossmatch result using a commercial gel tube crossmatch kit. Follow-up assessments in the reference laboratory showed that both cats of this report were Mik+, and neither showed any agglutination reactions in crossmatch tests against initial and other donor cats. The discrepancy between the test results at the original hospital and the PennGen laboratory remain unexplained, but may include differences in testing protocols and the potential for nonspecific autoagglutination falsely interpreted as incompatibility. The

latter, however, is unlikely, since the autoagglutination saline control well on the patient's original pretransfusion AB typing Card was negative.

When blood incompatibilities are detected against unknown and high-frequency RBC antigens, which can even happen when using the same donor due to prior sensitization, compatible donors can still be found among relatives. Indeed, a littermate of Cat 2 in this report was located and found to be compatible with Cat 2 on both major and minor crossmatch test. After it was recognized that the beneficial effects of the transfused canine RBC had been very transient, a unit from this littermate cat was transfused on an emergency basis and rapidly stabilized this critically ill and anemic patient.

Adverse transfusion reactions can be divided into immunologic and nonimmunologic reactions, and both have been reported in cats.[24] Reactions may occur with as little as 1 mL of blood [25], as observed with Cat 2 of this report. The AB and other blood group incompatibilities are the most feared immunologic reactions, and can typically be identified by typing and crossmatching. Other immunologic reactions can be due to reactions to white blood cell antigens as well as various plasma components. Other hemolytic but nonimmunologic reactions may be caused by bacterial contamination of units during collection, processing and storing of blood products.[26,27] Nonimmunologic reactions also include citrate toxicity, volume overload, and infectious disease transmission from the donor.[5,28,29]

Frequently, the precise cause of a transfusion reaction remains unknown, as in the case of Cat 2 of this report. When receiving blood from the original feline donor, Cat 2 exhibited an immediate severe reaction, which was very comparable to the reaction observed in cats following transfusion of incompatible blood (for example, transfusion of type A blood to a type B cat)[30,31], but the reactions could also have been caused by other immunologic reactions. Blood type incompatibility was initially suspected, but follow-up crossmatch studies could not confirm this, as no alloantibodies could be detected. There was no evidence of hemolysis in Cat 2 after transfusion with feline blood, but as only 1 mL of blood was transfused, the amount of released hemoglobin might not have been detectable. A complete follow-up investigation was not performed to determine the cause, but whenever severe acute reactions consistent with blood group incompatibility are observed, transfusion should be immediately discontinued and fluids, antihistamines and glucocorticosteroids must be administered as supportive care.

Xenotransfusions with canine blood have been previously used in anemic cats, when feline blood was not available[10-12,15] or not compatible, as illustrated in the case reports described

here. Cat 1 had discordant typing results and therefore received canine pRBC. However, this AB cat could have been safely transfused with type A pRBC or even whole blood if AB pRBC were not available, because type A cats rarely have strong alloantibodies.[2, 29-31] Moreover, a major crossmatch with a type A donor could detect any anti-A alloantibodies. Cat 2 experienced an acute transfusion reaction and initial crossmatches were incompatible, although subsequent testing could not confirm this. Cat 2 could potentially have been safely transfused with type A blood from another donor cat and not just the littermate.

As observed in other rare case reports and series [8-11,15], both cats in this report initially recovered uneventfully following canine xenotransfusion. However, as in other studies, both cats also experienced hemolysis and rapid clearance of canine RBC within 4 days.[32] A rapid return of the PCV to a pretransfusion level and evidence of intravascular hemolysis are indicative of an acute hemolytic transfusion reaction. In support, our ability to use the control band on the DEA 1 Strip to follow the canine RBC in the transfused cats revealed that transfused cells disappeared by Day 4. The control band on the DEA 1 Strip binds to a glycophorin present on canine but not feline RBC. This observation may be helpful for other cases in order to differentiate between lysis of patient's RBC caused by an underlying disease or trigger, and lysis of canine RBC following xenotransfusion. Worth noting are the different sequels to intravascular hemolytic transfusion reactions between animals and people. In cats and dogs, intravascular hemolysis neither causes acute kidney injury nor major vascular compromise in A-B and other mismatched transfusions or xenotransfusions.[17,33-37] Alternatively, when compatible feline blood is not available for an anemic cat, a highly purified polymerized bovine hemoglobin-based oxygen carrier (eg, Oxyglobin*, Dechra Pharmaceuticals* PLC, *Headquarters,* Northwich, UK*)* may be considered as an alternative to canine xenotransfusion, since it can improve the clinical signs of anemia for at least 24 h and is not associated with exposure of the patient to harmful membranes and other components from lyzed canine erythrocytes.[16]

The rapid disappearance of canine RBC following xenotransfusion into cats suggests that naturally occurring alloantibodies against canine RBC are present in cats. In order to investigate this further, we utilized currently available typing and crossmatch tests to detect antigens and alloantibodies. Feline blood samples typed with the DEA 1 Strip revealed a usually faint and rarely stronger band at the DEA 1 location, but never a positive control band. In the 2 anemic cats in this case report, the band at DEA 1 was initially not appreciated, likely due to the relative absence of feline RBC. However, after recovery, when feline RBC had been replenished, both showed a slight DEA 1 band similar to other cats. As the DEA 1

protein recognized by anti-DEA 1 in dogs is not known, it is unclear if cats have this antigen or if the antibody cross-reacts with another protein.

In contrast, canine blood does not react with the A, B and control agent on the AB Strip, but always shows positive Card agglutination reactions in the type B well and no reaction in the type A well. The monoclonal antibodies used in Strip and Card test are different. Mixing canine and feline blood produced the expected patterns: feline blood mixed with DEA 1-canine blood, showed a moderate DEA 1 band and a positive control, or a positive control and weak agglutination in the patient well with the Card.

Earlier experimental studies with transfusion of canine blood into feline patients suggested initial compatible major crossmatches with incompatible crossmatch agglutination reactions after $\geq$ 8 days.[8,9,13,] In the crossmatches reported here, however, we found that some cats had compatible major crossmatch results to nearly all dogs, while other cats exhibited incompatible results to nearly all dogs. These patterns seemed independent of the cats' AB blood type and dogs' DEA 1 type. Furthermore, most canine plasmas caused agglutination reactions with feline RBC. These studies indicate the presence of alloantibodies in some feline and nearly all canine plasma samples against the other species' RBC. The observed in vivo acute hemolytic reactions indicate the presence of alloantibodies against canine RBC in at least some cats, even if crossmatch results suggest compatibility. These alloantibodies may be hemolysins (rather than agglutinating antibodies), which were not specifically tested for in this and other studies.

Beside the RBC-related incompatibilities, considerations may also be given to heterocompatibility of plasma transfusions. Cat 1 of this report had a severe coagulopathy, presumably due to an anticoagulant rodenticide poisoning, and could also have benefited from plasma with coagulation factors, but only received canine pRBC and vitamin K_1. The potential benefits of xenotransfusion of cats with canine plasma is unknown, albeit early xenotransfusion studies used canine whole blood, and even pRBC contain some plasma (~10%). In fact, hemophilic human patients who develop inhibitors (antibodies) against human factor VIII can be sometimes successfully treated with heterologous porcine coagulation factors.[38,39] It is therefore, conceivable that canine coagulation factors could be active in vivo and interact with feline coagulation factors to enhance fibrin formation and hemostasis.

Finally, while there seem to be hemolytic incompatibility reactions seen in every transfused cat receiving canine blood, the acute fatal reactions observed following repeat

xenotransfusions in cats may not be explained solely by alloantibodies, but may also be related to plasma components, and may represent serum sickness.[40]

In conclusion, these 2 cases and our blood typing and compatibility study reiterate that xenotransfusions should only be considered in dire emergency situations, since canine blood causes incompatibility reactions in cats and is extremely short -lived.

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